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TITLE: **Use of GDNF-Releasing Nanofiber Nerve Guide Conduits for the Repair of Conus Medullaris/Cauda Equina Injury in the Nonhuman Primate**

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14. ABSTRACT
The primary goal of this collaborative research project is to translate a cauda equina injury and repair model from the rodent to the non-human primate to determine whether nanofiber nerve guidance conduits (NGCs), which release glial cell-line derived neurotrophic factor (GDNF), may be used to bridge tissue gaps between the spinal cord and avulsed ventral roots to promote neuroprotection, axonal regeneration, and functional reinnervation of peripheral target muscles.
In the first year of the work, we developed an improved version of the nanofiber NGCs with increased surface area of nanofibers and gradient loading of the GDNF. In the current second year, we manufactured nanofiber NGCs of different sizes and shipped them to Dr. Havton to be used in primate surgeries.

15. SUBJECT TERMS
GDNF-Releasing Nanofiber Nerve Guide Conduits; Conus Medullaris/Cauda Equina Injury

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Introduction:
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Body:
As stated in the SOW, the primary work at the Johns Hopkins University site is to:

> Design and fabricate GDNF-releasing and control nanofiber nerve guidance tubes in collaboration with Dr. Hai-Quan Mao and colleagues at Johns Hopkins Medical Center. The nerve guidance tubes will be customized in caliber and length to be suitable for use in non-human primate studies.

To this end, in the first year we have optimized our nanofiber nerve guidance conduits (NGCs). We have developed two new approaches to optimize the efficiency of the nanofiber NGCs:

Conduit design to increase nanofiber surface area within the luminal space

We have developed a new configuration/method to load the biodegradable fibers to the lumen of the nerve guidance conduits (NGCs) as shown in Fig. 1. This new configuration aims to increase the available surface area of aligned fibers to provide even more directional guidance to the regenerating axons. With our previous-generation design, longitudinally aligned fibers lined only the innermost surface of the conduit wall. This new nanofiber NGC design provides a greater number of fibers and spaces the fibers throughout the luminal space of the NGC by building the conduit from the inside-out. Briefly, thin films of gelatin are first cast into a rectangular shape of 15-mm by 30-mm. Next, aligned PCL nanofibers are deposited onto the gelatin films via electrospinning so that the fibers are aligned upon the short axis. The films are crosslinked with glutaraldehyde and wrapped tightly around a 500-micron mandrel so that the fibers remain oriented in the same direction as the mandrel. The film is then wrapped so that the film/fibers form several layers around the mandrel, to a final outer-diameter of about 1.5 mm. The wrapped mandrel is then covered with a random mesh of PCL nanofibers via electrospinning to generate suitably thick but porous conduit walls to protect the regenerating nerve. When viewed from a cross-section, the conduit is filled with a “spiral“

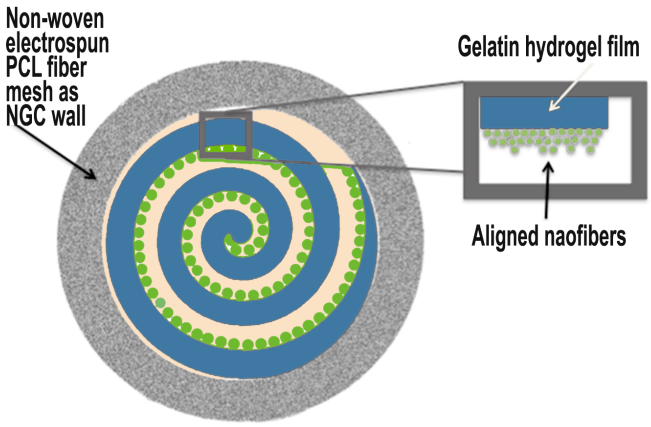

Fig. 1. Schematic illustration of the nanofiber distribution in the nerve guidance conduit. A cross-sectional view is shown here.

of aligned fibers. With this design, the surface area of aligned fibers within the 1.5 cm-long conduit increases by 6.4-fold, from 70.7 mm^2 to 450 mm^2.

Gradient neurotrophic factor loading to maximize the chemotractic cue

Instead of creating a uniform neurotrophic factor concentration in the hydrogel layer and NGC, we opted to develop a loading method to create neurotrophic factor gradients with higher neurotrophic factor loading at the distal end and lower loading at the proximal end (Fig. 2). Such a loading configuration will ensure that the released neurotrophic factor remains in effective concentration range by the time the regenerated axon reaches the distal end; it also mimics the situation where neurotrophic factors are released from the Schwann cells in the distal stump after injury, possibly creating a gradient chemotactic cue to guide axonal regeneration. Using a new method developed by the Khademhosseini lab at MIT, we have generated gelatin hydrogels containing multi-centimeter long GDNF gradients of controllable length, concentration range and steepness (Fig. 3). GDNF gradients are generated through passive flow of a GDNF solution through a PDMS stamp containing a 4-cm long channel containing an inlet and an outlet at opposite ends of the channel. Fig. 3 shows three gradients of fluorescently- labeled GDNF established by this method, each of which was generated using a different concentration range of GDNF. The differences in concentration ranges between the gradients is created by varying either the GDNF concentration of the inlet solution or by adding GDNF to the solution used to pre-fill the channel. Changing the GDNF concentration in the pre-fill or inlet solutions can change not only the concentration range of the gradient but also the steepness of the gradient. Gradient characteristics can also be controlled through variation of other parameters such as time between inlet injections and number of injections (data not shown). With such a high level of control for GDNF

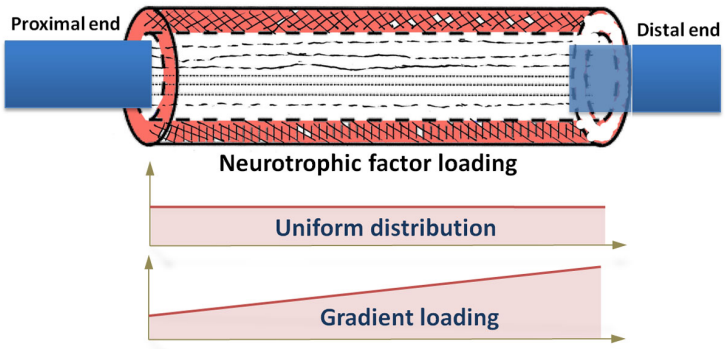

Fig. 2. Schematic diagram showing gradient loading of neurotrophic factor loading vs. uniform distribution.

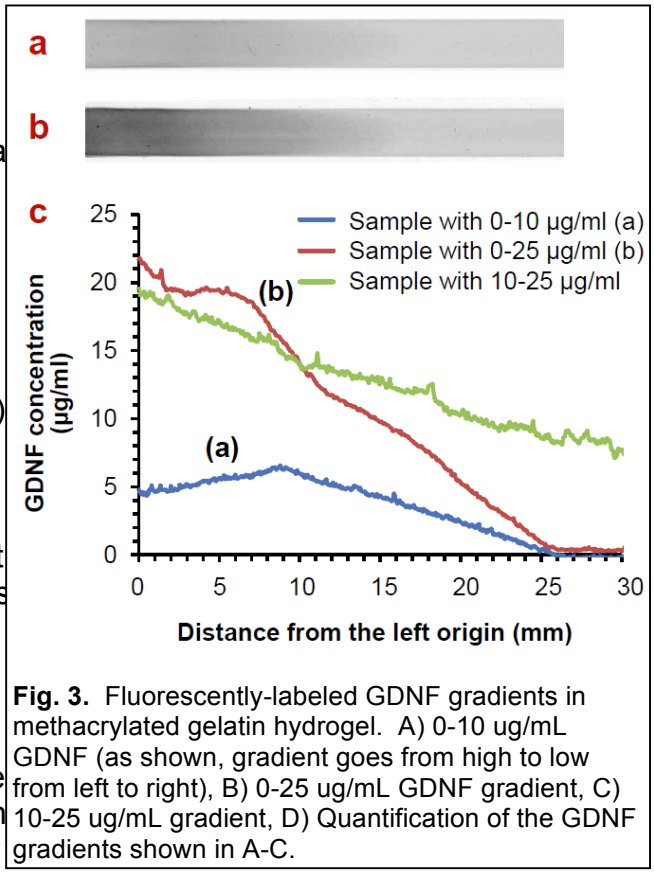

Fig. 3. Fluorescently-labeled GDNF gradients in methacrylated gelatin hydrogel. A) 0-10 ug/mL GDNF (as shown, gradient goes from high to low from left to right), B) 0-25 ug/mL GDNF gradient, C) 10-25 ug/mL gradient, D) Quantification of the GDNF gradients shown in A-C.

gradient. Additionally, the gradient-containing hydrogel produced by this method is mechanically robust enough to be handled by hand, allowing for this system to be incorporated into our new NGC design as shown in Fig. 1 for *in vivo* application.

In our second year, we manufactured nanofiber NGCs in various sizes, suitable for use in non-human primates. We prepared nanofiber NGCs of various diameters to match the differences in diameter of ventral nerve roots. Both control nanofiber NGCs without GDNF and the experimental groups with GDNF loading have been manufactured and shipped to Dr. Havton's laboratory. These were used in surgeries as described in his progress report.

Key Research Accomplishments:
Year 1: Refinement of the nanofiber NGCs
Year 2: Manufacture of nanofiber NGCs with and without GNDF for use in non-human primate surgeries by Dr. Havton

We have presented an abstract and poster at the 2012 Military Health System Research Symposium that was held August 13-16, 2012, in Fort Lauderdale, FL. The title of our presentation was: "Use of GDNF-Releasing Nanofiber Nerve Guide Conduits for Repair of Conus Medullaris/Cauda Equina Injury in the Non-Human Primate" by L.A. Havton, J.H. Nieto, M. Ohlsson, H.H. Chang, H.Q. Mao, A Höke, and K.L. Christe
All participating Principal Investigators, i.e. Dr. L.A. Havton, Dr. K.L. Christe, and Dr. A. Höke were present and participated in the MHSRS/ATACCC meeting in Fort Lauderdale.

Reportable Outcomes
No reportable outcomes yet.

Conclusion:
We have improved upon on the original design of the nanofiber NGCs and manufactured nanofiber NGCs with and without GNDF to be used in non-human primate models of cauda equina type of spinal injury by our collaborator, Dr. Havton. Animals that have been operated on are undergoing long-term evaluation to test whether the new design of nanofiber NGCs improve the outcomes from spinal cord injury.

References:
None

Appendices:
None